Open Literature Review Summary

Chemical Name: Imidacloprid

CAS No: 138261-41-3

PC Code: 129099

ECOTOX Record Number and Citation: E159245

Pettis, J. S., D. vanEngelsdorp, J. Johnson and G. Dively. 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema. Naturwissenschaften 99():153-158 Published online; 13 January 2012. DOI 10.1007/s00114-011-0881-1

Purpose of Review (Note: DP Barcode required for Quantitative studies):

Clothianidin Petition Evaluation

DP Barcode 408766

Date of Review: 04/04/2013

Summary of Study Findings:

According to the study authors, honey bee colonies were exposed to sublethal "doses" of imidacloprid during three brood generations (May – July) then subsequently challenged newly emerged bees with the gut parasite Nosema and infections increased significantly in bees from pesticide-treated coloies compared to controls demonstrating an indirect effect of pesticides on pathogen growth in honey bees. The authors claim to have demonstrated an increase in pathogen growth in individual bees with undetectable levels of imidacloprid from colonies exposed to imidacloprid. The authors assert that the "*interactions between pesticides and pathogens could be a major contributor to increased mortality of bee colonies, including colony collapse disorder, and other pollinator declines worldwide.*" Although the authors speculate on the broader implications of their research, they acknowledge that the increased Nosema loads identified in individual bees exposed to spores under laboratory conditions were not observed in the colonies from which the bees were obtained and that actual spore counts in colonies after the 10-wk study indicated higher average spore loads in controls (4.3 million) and the lowest average (0.5 million) in the 20 ppb treatment. The increased susceptibility of newly emerged bees to Nosema did not appear to be dose dependent given that roughly similar spore loads were observed in bees derived from colonies exposed to imidacloprid at either 5 or 20 ppb regardless of the strength of the inoculum used to expose bees.

Objective: To test the hypothesis that bees exposed to sub-lethal levels of imidacloprid are more susceptible to disease. Honey bee colonies were exposed to sub-lethal levels of imidacloprid and the newly emerged workers from these colonies were then challenged with the gut parasite *Nosema* spp.

Methods: Each colony was established in April 2008 using package bees (1.8 kg) and new equipment including frames with wax-coated foundation. All queens came from the same genetic source and colonies were managed to limit the levels of other pests/pathogens. A total of 30 colonies were divided into 3 treatment groups consisting of 10 colonies each. Colonies were further divided into 5 apiaries approximately 0.5 km apart containing 2 colonies from each of the treatments (6 colonies per apiary). Colonies were fed sucrose solution until natural forage became available in May.

Treatments consisted of untreated Megabee® protein patties (100 g) containing 0, 5 or 20 ppb imidacloprid. Imidacloprid was first made up in sucrose before being mixed with the Megabee® protein. Beginning in May each colony received four 80-g patties per week for 10 weeks. Unconsumed patties were removed after 7 days, weighed to determine consumption, and then replaced with new treatment patties. One week after the 10-wk feeding period, newly emerged adult bees (>5 g) and random-aged bees and protein patties from each treatment group along with stored bee bread (honey/pollen) from each treatment group were collected for imidacloprid residue analysis using GC/MS with a limit of detection (LOD) of 0.1 ppb.

Full-sized colonies (30 – 40,000 adults) were continually exposed to 5 or 20 ppb imidacloprid by provisioning the colonies with the protein supplement patties spiked with the compound. After 5 weeks of exposure (representing between 1.5 to 2.5 generations of bees during this exposure period) wax combs where taken into the laboratory where newly emerging adults from selected colonies were removed and placed into cages (3 from control colonies, 2 from colonies fed 5 ppb and 4 from colonies fed 20 ppb) containing 30 bees for Nosema challenge while an additional 20 newly emerged bees were weighed to determine average bee weight. A Nosema spore suspension was made by macerating the mid-guts of 10 worker bees (taken from a Nosema-infected colony) in 10 mL of water, centrifuging the suspension, then resuspending the resulting pellet in a 50% sucrose solution. Bees were then fed 10 mL of the suspension of *N. apis* and *N. ceranae* spores (~1 million spores/mL) over the first 2 days of their adult life and representing an individual bee dose of approximately 333,333 spores. After 10 days (12 days post-emergence), bees were sacrificed and the development of Nosema infection in individual bees determined.

A second trial was initiated after 8 weeks of exposure and contained cages of 10 bees (3 from each treatment); however, bees from different colonies than were used in the first trial were exposed to 10 mL of sugar solution containing 0, 0.1 or 1 million spores/mL in order to determine the potential effects of inoculum dose.

Results: Daily protein patty consumption did not differ significantly between treatments and averaged 29±0.84, 29.3±0.78 and 31.1±0.85 g in the control, 5 and 20 ppb colonies. Residues of imidacloprid were measured in bee bread and from random-aged bees collected from the colonies (**Table 1**); no imidacloprid was detected in newly emerged bees. Traces of imidacloprid were detected in bees and bee bread collected from control colonies. The weight of newly emerged bees from the 20 ppb treatment was significantly ($p<0.05$) less than control in the first trial; however, there was no significant different in emerged bee weight for the second trial.

Table 1 Levels of imidacloprid in bee bread (stored pollen/protein patties) and random-aged worker bees from experimental colonies and average weights of newly emerged bees from the three colony treatments for trials 1 and 2 in July and August 2008, respectively (n=20 bees/hive)

	Imidacloprid levels[a] (mean±SEM, ppb)			Emerged bee weight (mean±SEM, n=20 bees[d])	
Treatment colonies	Bee bread from colonies[b]	Random-aged bees from colonies[b]	Imid. level emerged bees[c]	Emerged bee weight (g) July trial 1	Emerged bee weight (g) Aug. Trial 2
Control	0.20±0.22	0.6±0.31	Not detected	0.115±0.0014	0.116±0.0018
Low 5 ppb	1.62±0.68	1.58±0.68	Not detected	0.112±0.0016	0.116±0.0017
High 20 ppb	3.49±1.55	3.67±1.48	Not detected	*0.106±0.0017	0.116±0.0020

*$P<0.05$ (indicate significant differences in emerged bee weights when compared to control hives in the same trial)

[a] Limit of detection is 0.1 ppb

[b] Sample size n = 10 patties sampled

[c] Newly emerged bees from both trials were tested for imidacloprid

[d] One control cage, July trial, had only 11 bees available to weigh, all others n=20 bees

According to the study authors, mortality in cages of newly emerged bees averaged less than 20% after 12 days in both phases of the study. Spore suspensions used in both trials consisted mainly of *N. ceranae*; however some *N. apis* was also present.

In the first trial, the unbalanced numbers of cages from the different treatment groups resulted from inconsistent emergence of adult bees from selected brood comb. According to the study authors, bees fed either 5 or 20 ppb had significantly ($p=0.0013$) higher spore loads compared to controls (**Figure 1**; reproduced from Pettis *et al.* 2012). In the second trial where bees were treated with increasing inoculums of spores, the authors state that there was an increasing spore level in the bees ($p<0.0001$), there was no difference in final spore counts in the bees after 12 days (**Figure 2**; reproduced from Pettis *et*

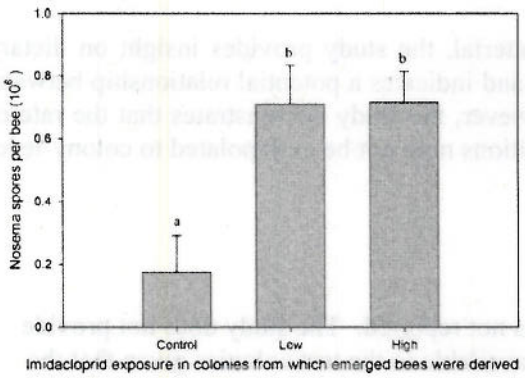

Fig. 1 Total *Nosema* spore count in 12-day-old bees derived from colonies feed high, low, and no levels of imidacloprid (July, trial 1). Immature bees were removed from colonies, allowed to emerge, and feed sugar solution with 10^6 *Nosema* spores per milliliter of sugar syrup. *Columns with different letters* are significantly different from each other (Tukey HSD test $P<0.05$)

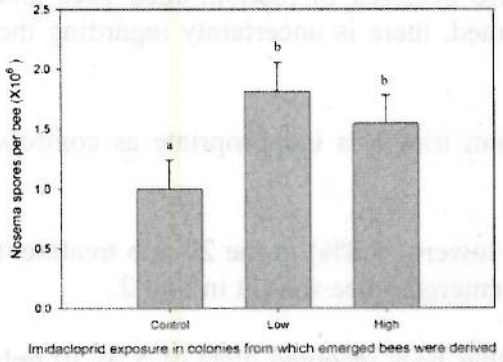

Fig. 3 Total *Nosema* spore count in 12-day-old bees derived from colonies feed high, low, and no levels of imidacloprid (August, trial 2). Immature bees were removed from colonies, allowed to emerge, and feed sugar solution with known amounts of spores (see text for details). *Columns with different letters* are significantly different from each other (Tukey HSD test $P<0.05$)

al. 2012). Therefore, the authors combined the data and then compared spore loads from trial 2 to the control from trial 1. Bees in trial 2 had significantly ($p<0.0001$) higher spore counts than the trial 1 control (**Figure 3**; reproduced from Pettis *et al*. 2012).

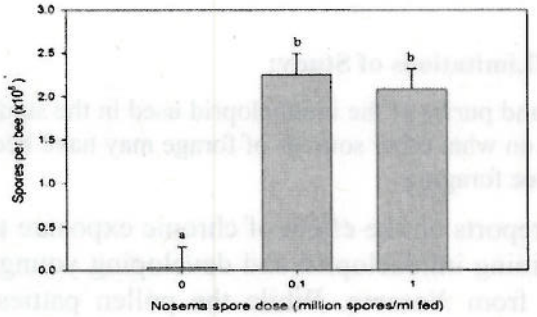

Fig. 2 Total spore count in 12-day-old bees fed two different levels of *Nosema* spores following adult emergence. *Columns with different letters* are significantly different from each other (Tukey HSD test $P<0.05$)

The authors note that of the group of 30 colonies that were used to provide bees for the cage studies, only three tested positive for Nosema and were excluded from used. After the 10-wk study period, 8 (3 control, 3 at 5 ppb and 2 at 20 ppb) of the 30 colonies tested positive for Nosema; however, there was no relationship between Nosema infection and imidacloprid treatment. Spore counts per bee averaged 4.3, 2,9 and 0.5 million in the 0, 5 and 20 ppb colonies.

The authors discuss other studies demonstrating a "synergism" between pesticides and Nosema and they assert that the current study "*clearly demonstrates that such interactions are possible in the real world, not just in the laboratory setting*" and that the interactions observed in their study "*could be a major contributor to increased mortality of honey bee colonies worldwide*".

Description of Use in Document (QUAL, QUAN, INV):

Qualitative

Rationale for Use:

Although there was control contamination with the test material, the study provides insight on dietary exposure of bee colonies to sublethal levels of imidacloprid and indicates a potential relationship between Nosema spore loads in newly emerged individual bees; however, the study demonstrates that the rate of infection observed on individual bees under laboratory conditions may not be extrapolated to colony-level susceptibility.

Comments/Limitations of Study:

The source and purity of the imidacloprid used in the study is not reported. The study does not provide information on what other sources of forage may have been available to the test colonies given that the bees were free foraging.

The paper reports on the effect of chronic exposure to imidacloprid. Adult bees were exposed to diets containing imidacloprid and developing young from these bees were then challenged with the spores from *Nosema*. While the pollen patties decreased on weight, the study does not provide data to indicate that this was due to actual consumption (dosing). To some extent, the spiked pollen patties were also stored as bee bread in the colony given the level of imidacloprid detected in the comb. While imidacloprid residues were detected in random-aged bees and shows that at least some of the spiked pollen was consumed, there is uncertainty regarding the actual "dosing".

The comparison of bees treated in trial 2 to controls from trial 1 is inappropriate as controls should only be compared to concurrent treatments.

Although emerged bee weight was significantly ($p<0.05$) lower (~7.8%) in the 20 ppb treatment in trial 1 compared to controls, there was no difference in emergent bee weight in trial 2.

Based on the data from the two trials, regardless of whether bees received diets of 5 or 20 ppb imidacloprid, they expressed roughly a 4-fold increase in the number of spores relative to controls regardless of the strength of the inoculum. Therefore, the response does not appear to be related to dose. Although both trials indicated that spore counts were significantly higher in newly emerged caged bees from the 5 and 20 ppb colonies relative to controls, average spore counts in bees collected directly from the colonies were highest in the controls (4.3 million) compared to either the 5 ppb (2.9 million) or 20 ppb (0.5 million) treatments.

Controls in the study were contaminated with the test chemical, imidacloprid; however, the levels reported in bee bread (0.2±0.22 ppb) and in random-aged bees (0.6±0.31 ppb) were low compared to the LOD (0.1 ppb).

While the researchers collected observations on individual bees, they extended their results to the colony itself even though colonies did not express any sign of *Nosema* infection and appeared to be healthy. Therefore, the authors' assertion that the current study "*clearly demonstrates that such interactions are possible in the real world, not just in the laboratory setting*" is not supported since the effects observed in the laboratory with caged bees did not appear to extend to the colonies from which they were derived. Also, given that there was no reported difference in mortality between control and treated colonies and that infection of the intact colonies did not appear to be an issue, the authors'

assertion that such interactions "*could be a major contributor to increased mortality of honey bee colonies worldwide*" does not appear to be supported by the current study.

Primary Reviewer:
Thomas Steeger, Ph.D., Senior Science Advisor, Environmental Risk Branch 4, Environmental Fate and Effects Division, Office of Pesticide Programs Thomas Steeger 4/4/13

Secondary Reviewer (required if study results are used quantitatively):

Not applicable